Example 10

Monopolar Spindle Formation following Application of a KSP Inhibitor

[0388] Human tumor cells Skov-3 (ovarian) are plated in 96-well plates at densities of 4,000 cells per well, allowed to adhere for 24 hours, and treated with various concentrations of the pyridmidinone derivatives for 24 hours. Cells are fixed in 4% formaldehyde and stained with antitubulin antibodies (subsequently recognized using fluorescently-labeled secondary antibody) and Hoechst dye (which stains DNA).

[0389] Visual inspection reveals that the compounds caused cell cycle arrest in the prometaphase stage of mitosis. DNA is condensed and spindle formation is initiated, but arrested cells uniformly display monopolar spindles, indicating that there is an inhibition of spindle pole body separation. Microinjection of anti-KSP antibodies also causes mitotic arrest with arrested cells displaying monopolar spindles.

Example 11

Inhibition of Cellular Proliferation in Tumor Cell Lines Treated with KSP Inhibitors

[0390] Cells are plated in 96-well plates at densities from 1000-2500 cells/well of a 96-well plate and allowed to adhere/grow for 24 hours. They are then treated with various concentrations of drug for 48 hours. The time at which compounds are added is considered T_0. A tetrazolium-based assay using the reagent 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (U.S. Pat. No. 5,185,450) (see Promega product catalog #G3580, CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay) is used to determine the number of viable cells at T_0 and the number of cells remaining after 48 hours compound exposure. The number of cells remaining after 48 hours is compared to the number of viable cells at the time of drug addition, allowing for calculation of growth inhibition.

[0391] The growth over 48 hours of cells in control wells that had been treated with vehicle only (0.25% DMSO) is considered 100% growth and the growth of cells in wells with compounds is compared to this.

[0392] A Gi_{50} is calculated by plotting the concentration of compound in μM vs the percentage of cell growth in treated wells. The Gi_{50} calculated for the compounds is the estimated concentration at which growth is inhibited by 50% compared to control, i.e., the concentration at which:

$$100\times[(\text{Treated}_{48}-T_0)/(\text{Control}_{48}-T_0)]=50$$

wherein Treated_{48} is the value at 48 hours for the treated cells and Control_{48} is the value at 48 hours for the control population.

[0393] All concentrations of compounds are tested in duplicate and controls are averaged over 12 wells. A very similar 96-well plate layout and Gi_{50} calculation scheme is used by the National Cancer Institute (see Monks, et al., J. Natl. Cancer Inst. 83:757-766 (1991)). However, the method by which the National Cancer Institute quantitates cell number does not use MTS, but instead employs alternative methods.

[0394] The compound of Example 1 above inhibited cell proliferation in human ovarian tumor cell lines (SKOV-3).

Example 12

Calculation of IC_{50}

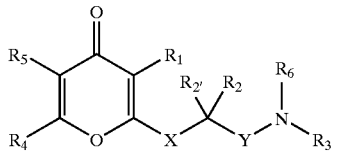
[0395] Measurement of a compound's IC_{50} for KSP activity uses an ATPase assay. The following solutions are used: Solution 1 consists of 3 mM phosphoenolpyruvate potassium salt (Sigma P-7127), 2 mM ATP (Sigma A-3377), 1 mM IDTT (Sigma D-9779), 5 μM paclitaxel (Sigma T-7402), 10 ppm antifoam 289 (Sigma A-8436), 25 mM Pipes/KOH pH 6.8 (Sigma P6757), 2 mM MgC12 (VWR JT400301), and 1 mM EGTA (Sigma E3889). Solution 2 consists of 1 mM NADH (Sigma N8129), 0.2 mg/ml BSA (Sigma A7906), pyruvate kinase 7 U/ml, L-lactate dehydrogenase 10 U/ml (Sigma P0294), 100 nM KSP motor domain, 50 μg/ml microtubules, 1 mM DTT (Sigma D9779), 5 μM paclitaxel (Sigma T-7402), 10 ppm antifoam 289 (Sigma A-8436), 25 mM Pipes/KOH pH 6.8 (Sigma P6757), 2 mM MgC12 (VWR JT4003-01), and 1 mM EGTA (Sigma E3889). Serial dilutions (8-12 two-fold dilutions) of the compound are made in a 96-well microtiter plate (Corning Costar 3695) using Solution 1. Following serial dilution each well has 50 μl of Solution 1. The reaction is started by adding 50 μl of solution 2 to each well. This may be done with a multichannel pipettor either manually or with automated liquid handling devices. The microtiter plate is then transferred to a microplate absorbance reader and multiple absorbance readings at 340 nm are taken for each well in a kinetic mode. The observed rate of change, which is proportional to the ATPase rate, is then plotted as a function of the compound concentration. For a standard IC_{50} determination the data acquired is fit by the following four parameter equation using a nonlinear fitting program (e.g., Grafit 4):

$$y=\frac{\text{Range}}{1+\left(\frac{x}{IC_{50}}\right)^{s}}+\text{Background}$$

where y is the observed rate and x is the compound concentration.

1. A method of inhibiting KSP which comprises contacting KSP with an effective amount of at least one chemical entity chosen from compounds of Formula I:

(Formula I)



and pharmaceutically acceptable salts thereof; wherein:

X is optionally substituted alkylene, —C(O)—, or is absent;

Y is optionally substituted alkylene, —C(O)—, or is absent;